

IN THE CLAIMS

Claims 1-11 (Cancelled)

12. (amended) A method for preparing a protein with a C-terminal thioester, comprising:

- (a) expressing a recombinant precursor protein in a host cell, the precursor protein comprising the protein fused to an intein and optionally a binding protein binding domain, the intein being selected from a naturally occurring intein, an intein derivative or an intein mutant, wherein the intein is capable of being thiol induced cleavage cleaved from the protein in the presence of 2-mercaptopethanesulfonic acid; and
- (b) contacting the expressed precursor protein with a thiol reagent 2-mercaptopethanesulfonic acid and inducing cleavage of the intein from the precursor protein so as to form the target protein having the C-terminal thioester.

13. (amended) The method according to claim 12, wherein the intein is selected from Sce Vma VMA intein and Mxe GyrA GyrA intein.

14. (amended) The method according to of claim 12, wherein the thiol reagent is selected from 2-mercaptopethanesulfonic acid, thiophenol, dithiothreitol, and 3-mercaptopropionic acid protein binding domain is a chitin binding domain.

15. (amended) The method according to claim 12, wherein the precursor protein is selected from a Bst DNA polymerase I large fragment, thioredoxin and or a cytotoxic protein.

16. (amended) The method according to claim 12, wherein the precursor protein is selected from a maltose binding protein and paramyosin.

17. (amended) A method for expressing a recombinant protein precursor, comprising:

inserting a nucleic acid sequence encoding a target protein into a plasmid at a multiple cloning site located upstream of and in frame with a fusion gene encoding an intein and a binding protein domain wherein intein is selected from a naturally occurring intein, and intein derivative and an intein mutant modified intein; and

(i) the intein is selected from a naturally occurring intein, an intein derivative or an intein mutant; and

(ii) the multiple cloning site contains a linker and the linker sequence is selected from SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3 or SEQ ID NO:4; and

introducing the plasmid into a host cell for expressing the recombinant precursor protein.

18. (previously amended) The method of claim 17, wherein the binding protein encoded by the nucleic acid is a chitin binding protein.

Claims 19-20 (cancelled)

21. (previously added) The method according to claim 17, wherein the plasmid is a pTXB plasmid.

22. (amended) A method of modifying a protein by ligating a synthetic peptide or synthetic second protein in vitro to the to an inactive protein so as to restore protein activity, comprising:

- (a) expressing in a host cell, the protein fused to one of an intein, an intein derivative or ~~an intein mutant intein~~ a mutant intein, wherein the intein is capable of thiol induced cleavage;
- (b) inducing intein mediated cleavage of the protein by adding a ~~thiol reagent~~ 2-mercaptoethanesulfonic acid so as to form a C-terminal thioester on the protein;
- (c) preparing a synthetic peptide or a synthetic second protein having an N-terminal cysteine; and
- (d) ligating ~~the inactive form of~~ the protein to the synthetic peptide or a synthetic second protein to modify the protein ~~activity~~.

23. (previously added) The method according to claim 22, wherein the protein prior to modification is a cytotoxic protein.

24. (amended) ~~the method of claim 21, wherein the cytotoxic protein is a restriction endonuclease~~ The method according to claim 22, wherein the intein, the intein derivative or the mutant intein in step (a) is optionally fused to a protein binding domain.

25. (amended) A method of labeling a target protein, comprising:

- (a) expressing a recombinant precursor protein in a host cell, the precursor protein comprising the target protein fused to an intein

and optionally a binding protein domain, the intein being selected from a naturally occurring intein, an intein derivative or an intein mutant, wherein the intein is capable of thiol induced cleavage;

- (b) cleaving the precursor protein in the presence of ~~a thiol reagent~~ 2-mercaptopethan sulfonic acid so as to form the target protein having a C-terminal thioester;
- (c) preparing a synthetic peptide or protein having a marker and an N-terminal cysteine; and
- (d) ligating the target protein with the synthetic peptide or protein for ~~labelling~~ labeling the target protein.

26. (previously added) The method according to claim 24 25, wherein the marker is selected from the group consisting of a fluorescent marker, a spin label, an affinity tag, and a radiolabel.

27. (previously added) The method according to claim 24 25, wherein the peptide fragment is an antigenic determinant.

28. (amended) A method for ligating a ~~first target protein with a second target protein, the method~~ synthetic protein or peptide to an inactive protein so as to restore protein activity, comprising

- (a) expressing in a host cell, a fusion protein comprising the first target protein fused to an intein having an N terminal cleavage activity wherein the fusion protein is expressed from a first plasmid at the C-terminus to one of an intein, an intein derivative or an intein mutant wherein the fusion protein is expressed from a plasmid;

- (b) ~~contacting the fusion protein of step (a) with a thiol reagent for inducing cleavage of the intein to produce a C terminal thioester on the first target protein; and inducing intein mediated cleavage of the protein by adding 2-mercaptopethanesulfonic acid so as to form a C-terminal thioester on the protein;~~
- (c) ~~combining a mixture for permitting ligation, the C-terminal thioester on the first target protein and a thioester reactive N-terminal amino acid on the second target protein preparing a synthetic protein or peptide having an N-terminal cysteine; and~~
- (d) ~~ligating the inactive form of the protein to the synthetic peptide to restore protein activity.~~

29. (previously amended) The method according to claim 28, wherein the protein is a cytotoxic protein.

30. (previously amended) The method of claim 29, wherein the cytotoxic protein is a restriction endonuclease.

Claims 31-33 (Cancelled)